

CLAIMS

1. DNA encoding at least one luciferase selected from the group consisting a red-emitting luciferase and a green-emitting luciferase derived from a rail road worm and a green-emitting  
5 luciferase and an orange-emitting luciferase derived from *Rhagophthalmus ohba* stably expressed in mammalian cells, characterized in that (1) the DNA has no binding sequence for an additional transcription factor in the mammalian cells and has a codon usage for the mammal.  
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2. The DNA according to claim 1, characterized in that the mammal is human and the DNA has at least one nucleotide sequence selected from the group consisting of SEQ ID NOS:7, 10, 11 and 16.
- 15 3. A method for enabling the expression of DNA encoding a luciferase derived from a rail road worm or *Rhagophthalmus ohba* in mammalian cells, characterized by having
  - 1) a step of altering a cDNA sequence such that no additional transcription factor is bound;
  - 20 2) a step of changing a codon usage for insects to that for mammals in the cDNA sequence; and optionally
  - 3) a step of altering the cDNA sequence with many restriction enzyme sites due to limited application at the use.
- 25 4. The method according to claim 3, characterized in that an amino acid sequence of the luciferase is not altered.
5. A polypeptide which is a luciferase with a maximum luminescence wavelength of 630 nm, represented by any of the  
30 followings:
  - (1) a polypeptide having an amino acid sequence of SEQ ID NO:4; and
  - (2) a polypeptide having one or more amino acid substitutions, additions or deletions in the sequence of SEQ ID  
35 NO:4.

6. The polypeptide according to claim 5, expressed in mammalian cells.

5 7. A gene construct incorporating one or two or more genes of luciferases which emit light whose wavelength does not substantially depend on a determining condition and maximum luminescence wavelength is 535 to 635 nm, to be stably expressible in mammalian cells.

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8. The gene construct according to claim 7 incorporating 3 or more luciferase genes stably expressibly in mammalian cells by incorporating one or two or more genes of luciferases with a maximum luminescence wavelength of 460 to 520 nm together with  
15 one or two or more genes of luciferases which emit light whose wavelength does not substantially depend on a determining condition and maximum luminescence wavelength is 535 to 635 nm.

9. The gene construct according to claim 7 wherein the above  
20 luciferase gene is a gene encoding at least one luciferase selected from the group consisting of a red-emitting luciferase and a green-emitting luciferase derived from a rail road worm and a green-emitting luciferase and an orange-emitting luciferase derived from *Rhagophthalmus ohba* stably expressed in mammalian  
25 cells.

10. The gene construct according to claim 7 comprising an element for promoting efficiency of translation and/or an element for stabilizing mRNA.

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11. A gene construct capable of distinctively determining each light emitted from two or more luciferases, by incorporating one or two or more genes of the luciferases which emit light whose wavelength does not substantially depend on a determining  
35 condition and if necessary a gene of the luciferase which emits

light whose wavelength is different and does not substantially depend on the determining condition under the control of different promoters.

5 12. An expression vector containing the gene construct according to any of claims 7 to 11.

13. Mammalian cells transformed with the gene construct according to any of claims 7 to 11 or the expression vector  
10 according to claim 12.

14. Mammalian cells stably expressibly incorporating two or more genes of luciferases which emit mutually distinct light whose luminescence wavelength does not substantially depend on a  
15 determining condition under the control of different promoters in the mammalian cells.

15. The mammalian cells according to claim 13 or 14 wherein two or more of the above luciferases have a maximum luminescence  
20 wavelength of 535 to 635 nm and can emit with one substrate.

16. The mammalian cells according to claim 15 comprising a red-emitting luciferase gene from a rail road worm and further comprising at least two or more selected from the group  
25 consisting of a green-emitting luciferase gene from the rail road worm, a green-emitting luciferase gene from *Rhagophthalmus ohba*, an orange-emitting luciferase from *Rhagophthalmus ohba*, and a blue-emitting luciferase gene under the control of different promoters.

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17. The mammalian cells according to claim 14 stably expressibly incorporating genes of three or more luciferases which emit mutually distinct light whose luminescence wavelength does not substantially depend on a determining condition under  
35 the control of different promoters in the mammalian cells.

18. The mammalian cells according to claim 14 having three or more luciferase genes under the control of different promoters wherein a first luciferase gene is under the control of a  
5 constantly expressed promoter, a second luciferase gene is under the control of a toxicity assessing promoter, and remaining one or more luciferase genes are under the control of a promoter subjected to assessment.

10 19. The mammalian cells according to claim 14 having three or more luciferase genes under the control of different promoters wherein a first luciferase gene is under the control of a constantly expressed promoter, a second luciferase gene is under the control of a pseudopromoter, and remaining one or more  
15 luciferase genes are under the control of a promoter subjected to assessment.

20. The mammalian cells according to claim 14 having 4 or more luciferase genes under the control of different promoters,  
20 wherein a first luciferase gene is under the control of a constantly expressed promoter, a second luciferase gene is under the control of a toxicity assessing promoter, a third luciferase gene is under the control of a promoter of a protein which accepts an external factor, and remaining one or more luciferase  
25 genes are under the control of a promoter subjected to assessment.

21. The mammalian cells according to claim 14 having 4 or more luciferase genes under the control of different promoters, wherein a first luciferase gene is under the control of a  
30 constantly expressed promoter, a second luciferase gene is under the control of a pseudopromoter, a third luciferase gene is under the control of a promoter of a protein which accepts an exogenous factor, and remaining one or more luciferase genes are under the control of a promoter subjected to assessment.

22. The mammalian cells according to claim 14 having two luciferase genes under the control of different promoters, wherein a first luciferase gene is under the control of a constantly expressed promoter, and a second luciferase gene is  
5 under the control of a toxicity assessing promoter.

23. The mammalian cells according to claim 14 having two luciferase genes under the control of different promoters, wherein a first luciferase gene is under the control of a  
10 constantly expressed promoter, and a second luciferase gene is under the control of a pseudopromoter.

24. A method for screening drugs including a step of culturing the mammalian cells according to any of claims 18 to 21 in the  
15 presence of a drug candidate compound in a medium of the mammalian cells, a step of quantifying an amount of the above luciferase in the presence or absence of the candidate compound, and a step of assessing an effect of the candidate compound on a promoter subjected to assessment, which is linked to at least one  
20 luciferase.

25. A system for multiply determining transcription activity of each promoter linked to each luciferase before and after a change of a culture environment by changing the culture environment of  
25 the mammalian cells according to any of claims 13 to 23, and assessing expressed amounts of two or more luciferases which emit mutually distinct light whose luminescence wavelength does not depend on a determining condition.

30 26. The system according to claim 23 for simultaneously determining expressed amounts of two or more luciferases.

27. The system according to claim 23 capable of determining expressed amounts of three or more luciferases.